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13. ABSTRACT (Maximum 200 Words)

The purpose of this research program is to contribute towards two major objectives in support of advancing our ability to prevent or treat bone failure or fragility:

1. Developing and characterizing methods of evaluating bone properties in animal models that goes beyond measures of bone density and global mechanical properties.
2. Evaluating the influence of physical forces and nutritional status on bone biomechanical integrity.

Specifically, it was the purpose of this study to apply a hierarchical approach to quantifying the properties of murine bone to the level of the extracellular matrix. Furthermore, the study was designed to test hypotheses concerning the interplay between vitamin D and calcium nutritional support and physical forces.

Progress during the third year has followed the proposed statement of work. 394 animals have been completed and nearly all the vertebra, femurs and tibias have been evaluated geometrically, morphologically and mechanically. Genetic strain has a significant influence on properties, but exercise has had little effect. Nutrition is also important. From a histologic perspective, the tissue constituency is unaffected, suggesting only geometric variations, not inherent material changes.

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A. Introduction

It is well known that the maintenance and adaptation of bone integrity is dependent on a complex interaction of metabolic and environmental factors (mechanical stresses, nutritional status). Unfortunately, the specific relationship between these factors and the biomechanical competence of bone tissue remains incompletely quantified. As a result, strategies for preventing or effectively treating bone fragility or enhancing general bone health are far from being optimized. The specific goals of this research program is to contribute to two major objectives in support of reducing the incidence of fracture:

a. The development and application of micro-imaging and testing techniques in animal models to study bone structure function properties.

b. Exploring the influence of calcium and vitamin D metabolism and physical forces on bone integrity.

B. Body

The progress of this research program is described below, as a function of the statements of work that were approved by the USAMRMC. The statement of work was proposed as follows:

1. The acquisition of DBP founder mice and breeding will be performed during year 1 and year 2 to produce 180 animals for testing.

2. Mechanical fabrication and calibration of all testing holders and test fixtures will be completed during the first 9 months of study. Maintenance, recalibration and replacement of parts will continue year 2 through year 4.

3. Micro CT, whole bone testing of DBP mice will be completed year 1 and year 2.

4. Microspecimen production and testing of DBP bones will be completed year 2 through year 3.

5. Micro CT, Whole bone testing of C57BL/6J and C3H/HeJ bones will be conducted year 1 through year 3.

6. Microspecimen testing of C57BL/6J and C3H/HeJ bone will be tested year 2.5 through year 3.5.

7. Raman imaging, SEM, and light microscopy of DBP mice bone will be conducted year 1 through year 3.

8. Raman imaging, SEM, and light microscopy of C57BL/J6 and C3H/HeJ bone will be tested year 2 through year 3.5.

9. Final data analyses and correlations across all groups will be completed during year 4.

Since many of the tasks were described as objectives to be completed over 1 to 4 years, the progress report can't follow these nine tasks precisely. Instead, we have presented the specific tasks that were proposed for completion during the third year. The tasks are outlined in **"bold"**, followed by a description of the accomplishments.

The acquisition of DBP founder mice and breeding will be performed to produce the 180 mice for testing

As noted in earlier progress reports, DBP breeder mice (4 females and 2 males) were obtained from Dr. Nancy Cooke at the University of Pennsylvania. These mice are being used to generate our own population of heterozygote breeders. Due to background noise, homozygous breeders cannot be used. We have also developed protocols for genotyping the newborn mice.
During the past year we have worked, feverishly to breed these mice, but have continued to run into some difficult problems. We lost most of our colony to an outbreak of MPV (Mouse Parvo Virus) that afflicted many areas in our institution. We also had some difficulties with infertility. We have risen above most of these problems and have the colony growing and being entered into the testing protocols. As a result we have done some adjustments in our overall schedule and completed additional testing activity on other mouse bones, thereby maintaining the overall excellent progress and providing some flexibility to complete the breeding and testing. We are confident that we will still meet our overall objectives in the program. The goal of examining the DBP mice has been rescheduled to be completed during the next 15 months.

Histologic assessment of the mouse bone constituency

As proposed, we have prepared and reviewed the histologic features of selected bone specimens from all of our animal groups evaluated to date. This analysis was performed to assess whether there were microanatomical or morphologic features that varied among the test groups. We prepared and analyzed 120 bones. These procedures went very well and we determined that there were no systematic variations in matrix content, cell morphology, cell matrix interactions and other features. An example of the histologic sections and review is illustrated in Figure 1.

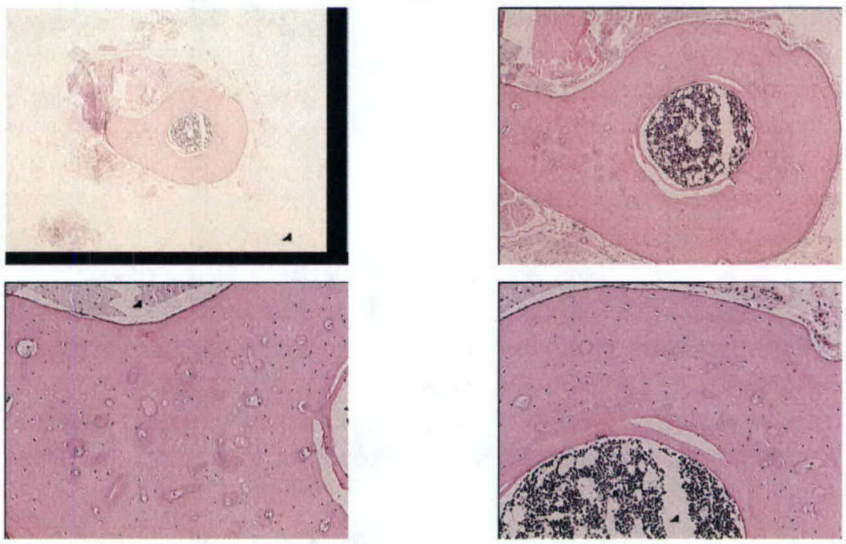


Figure 1: Example of histologic features of murine bone samples. No systematic differences were observed in any of the group.

Micro CT and whole bone testing of bones will be conducted year 1 through year 3

All acquisition, Micro CT and whole bone mechanical testing is now near completion for all the mice, excluding the DBP colony. This represents the largest portion of the proposed program and also represents the majority of progress for the program. We have only 240 femurs remaining to test, and all of the vertebral bodies have now been tested. The vertebral studies represent a substantial accomplishment for this period. By way of summary, we have completed the following:

A. Femoral Analyses

Studies	Micro CT scan	Micro CT analysis	Mechanical testing	Raman spectroscopy
Completed	392	392	128	30
Remaining to complete	0	0	240	100

B. Vertebral Body Analyses

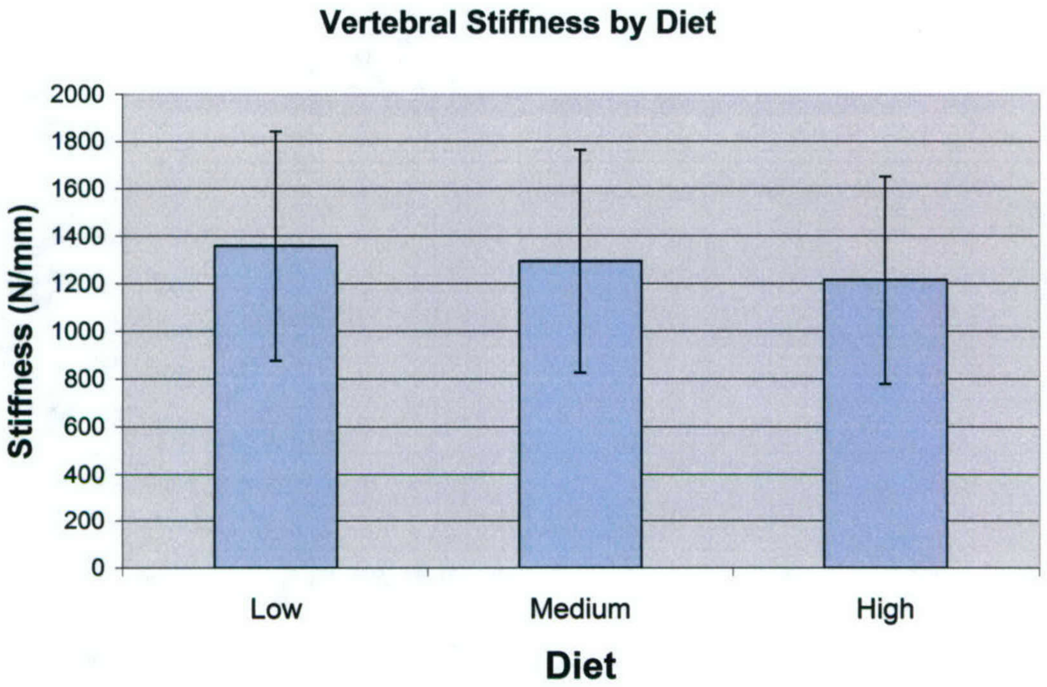
Studies	Micro CT scan	Micro CT analysis	Mechanical testing
Completed	382	381	394
Remaining to complete	0	0	0

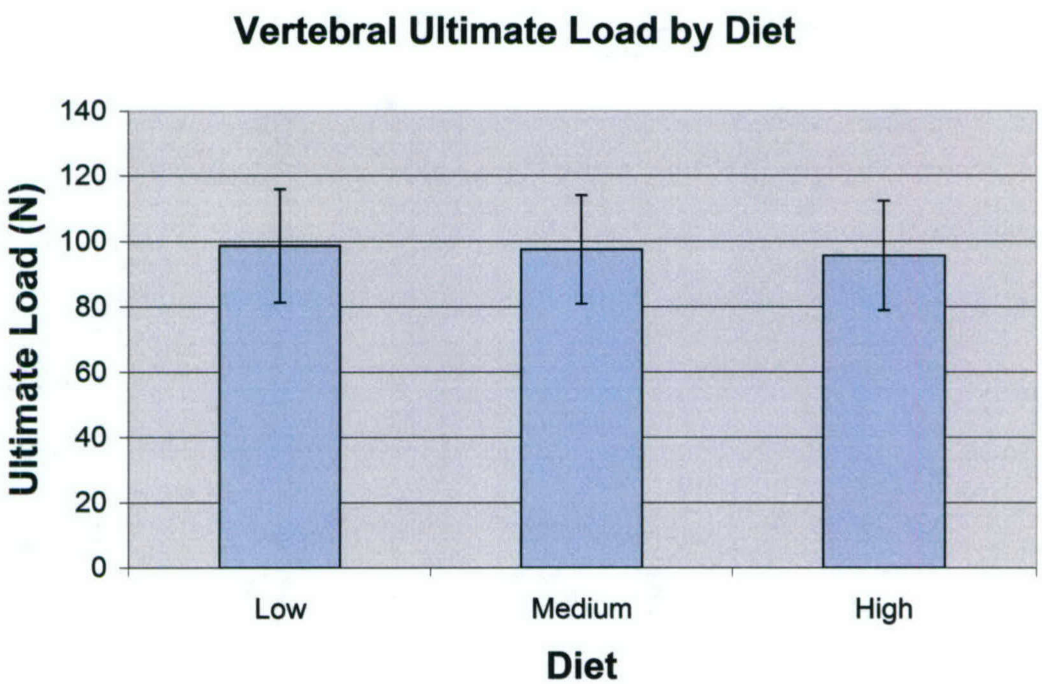
Microspecimen preparations

We have now completed the protocols for the preparation and machining of all of the microspecimens for ECM material property evaluations. This time consuming process has been optimized using the micro-milling system we have fabricated and associated dissecting microscope imaging. We have completed the machining of approximately 30 bone segments to date. We have also completed the validation of the micro-mechanical testing systems using both 4 point bending and nanoindentation.

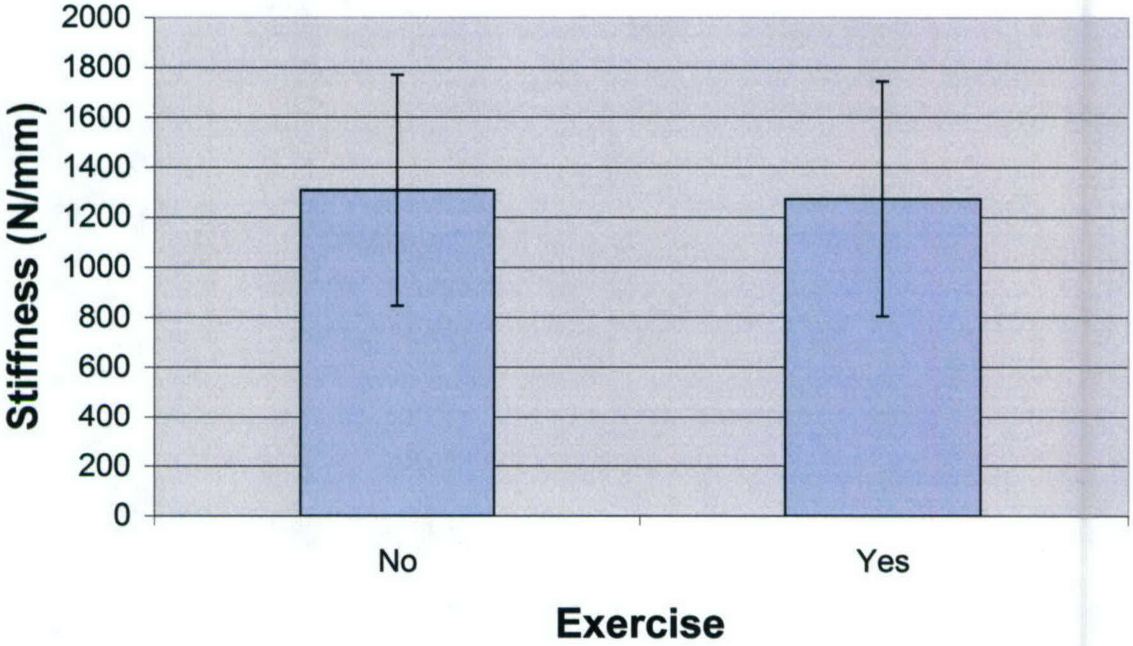
Example results for this period

The results from analysis of the vertebral body mechanical tests can be seen from the following graphs. These have been segmented into effects by diet and effects by exercise.

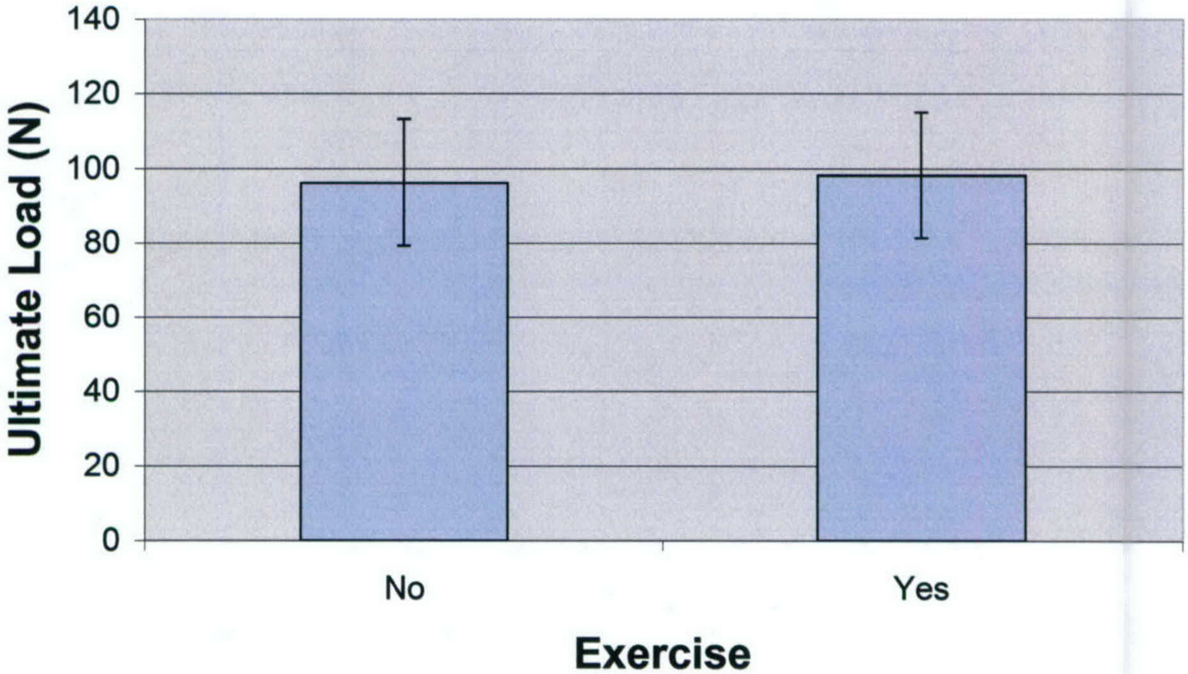

Vertebral Stiffness by Diet
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Stiffness (N/mm)
Low
Medium
High
Diet


Vertebral Ultimate Load by Diet
140
120
100
80
60
40
20
0
Ultimate Load (N)
Low
Medium
High
Diet

Vertebral Stiffness by Exercise



Vertebral Ultimate Load by Exercise



C. Key Research Finding

• Micro-milling and micro-testing systems fabricated are functioning and specimens are currently under production.
• 394 mice, which were entered into study to evaluate effects of gender, strain, nutrition and exercise on morphology and mechanical properties of bone, have been studied. All have been analyzed for vertebral mechanical and morphologic properties. All femoral specimens have been analyzed for morphology using Micro CT and 128 have been completed for mechanical testing.
• 120 tibial specimens have been prepared for histologic analysis. All have been sectioned, stained analyzed and documented using a Bioquant imaging system.
• DBP mice colony is being reconstituted and will be evaluated for properties during the next 15 months. These tasks have been rescheduled due to problems with breeding and a viral outbreak. Shifting of other tasks and procedures has been accomplished to maintain the overall schedule of the program.
• Mouse strain had a significant effect on geometric and preliminarily mechanical properties in the femurs. Strain had a significant effect on vertebral properties.
• Exercise did not have a significant effect on any of the femoral or vertebral mechanical whole-bone parameters.
• In vertebra, the normal dietary calcium group had a significantly higher bone volume fraction as compared to the low (p=0.003) and high (p=0.003) groups. The bone surface to volume ratio of the normal group was shown to be significantly lower than the low dietary calcium group (p=0.030), however no significant differences were seen between any of the other groupings.
• In vertebra, the normal dietary calcium group had a significantly higher trabecular thickness as compared to the low group (p<0.000) and marginally significant as compared to the high (p=0.051) group.
• Exercise has had no effect on the parameters tested to date. It may be possible that the material properties are affected, but we would hypothesize that these parallel the whole bone properties.

D. Reportable Outcomes

Kriegl JM, Oyserman S, Roller SA, Blumenfeld J, Volkman SK, Nashi S, Hall JM, McCreadie BR, Goldstein SA: Influences of nutrition and physical forces on bone structure/function properties. Trans ORS 50th Annual Meeting, No: 0393, March 7-11, 2004, San Francisco, CA.

E. Conclusions

The third year of work has been very successful and productive, despite the setback with DBP breeding. We have entered and analyzed a very large number of animals in to the study and have maintained the timetable originally proposed in the program. The first data of femoral and vertebral bone demonstrate significant effects of mouse strain and dietary calcium levels. The studies have also demonstrated the ability of the micro-imaging and testing protocols to determine the effects of a variety of factors on bone structure and function properties.

F. References

None

G. Appendices

1. Mouse census database for all animals entered into the studies.

2. Abstract

Appendix 1

Database documenting the animals entered into the studies and progress

									Femur				Vertebrae				Tibia	
Mouse ID	Cage ID	Group	Mark	Sex	Strain	DOB	Diet	Exercise	Scan	Analy.	Mech.	Raman	Scan	Analy.	Mech.	Analy.	Histology	Re-fill
2	1a	1a	R	M	C57	4/21/02	Low	Yes	1	1	1	0	1	1	1	1	1	0
9	1b		L	M	C57	4/21/02	Low	Yes	1	1	1	0	1	1	1	1	0	1
16	1c		B	M	C57	4/21/02	Low	Yes	1	1	1	0	1	1	1	1	0	0
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37	2		L	M	C57	4/21/02	Low	No	1	1	1	0	1	1	1	1	0	0
44	2		B	M	C57	4/21/02	Low	No	1	1	1	0	1	1	1	1	0	0
51	2		N	M	C57	4/21/02	Low	No	1	1	1	0	1	1	1	1	0	0
58	3	1a	R	F	C57	4/21/02	Norm	Yes	1	1	1	0	1	1	1	1	0	0
65	3		L	F	C57	4/21/02	Norm	Yes	1	1	1	0	0	0	1	1	0	0
72	3		B	F	C57	4/21/02	Norm	Yes	1	1	1	0	1	1	1	1	0	0
79	3		N	F	C57	4/21/02	Norm	Yes	1	1	1	0	1	1	1	1	0	0
86	4	1b	R	F	C57	4/21/02	Norm	No	1	1	1	0	1	1	1	1	0	0
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170	7	1a	R	M	C3	4/21/02	Low	Yes	1	1	1	0	1	1	1	1	0	0
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Database documenting the animals entered into the studies and progress

Mouse ID	Cage ID	Group	Mark	Sex	Strain	DOB	Diet	Exercise	Scan	Analy.	Mech.	Raman	Scan	Analy.	Mech.	Analy.	Histology	Re-fill
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296	11		B	M	C3	4/21/02	High	Yes	1	1	1	0	1	1	1	1	1	0
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Database documenting the animals entered into the studies and progress

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632	23		B	F	C3	4/28/02	High	Yes	1	1	1	0	1	1	1	1	0	1
639	23		N	F	C3	4/28/02	High	Yes	1	1	1	0	1	1	1	1	1	0
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660	24		B	F	C3	4/28/02	High	No	1	1	1	0	1	1	1	1	0	0
667	24		N	F	C3	4/28/02	High	No	1	1	1	0	1	1	1	1	0	1
674	25a	3a	R	M	C57	7/17/02	Low	Yes	1	1	1	0	1	1	1	1	0	0
~~681~~	~~25b~~		~~L~~	~~M~~	~~C57~~	~~7/17/02~~	~~Low~~	~~Yes~~	0	0	0	0	0	0	0	0	0	0
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~~695~~	~~25d~~		~~N~~	~~M~~	~~C57~~	~~7/17/02~~	~~Low~~	~~Yes~~	0	0	0	0	0	0	0	0	0	0
702	26	3b	R	M	C57	7/17/02	Low	No	1	1	1	0	1	1	1	1	0	0
709	26		L	M	C57	7/17/02	Low	No	1	1	1	0	1	1	1	1	1	0
716	26		B	M	C57	7/17/02	Low	No	1	1	0	0	1	1	1	1	0	0
723	26		N	M	C57	7/17/02	Low	No	1	1	1	0	1	1	1	1	0	0
730	27	3a	R	F	C57	7/17/02	Norm	Yes	1	1	1	0	1	1	1	1	0	0
737	27		L	F	C57	7/17/02	Norm	Yes	1	1	1	0	1	1	1	1	0	1
744	27		B	F	C57	7/17/02	Norm	Yes	1	1	1	0	1	1	1	1	0	0
751	27		N	F	C57	7/17/02	Norm	Yes	0	0	0	0	1	1	1	1	1	0
~~758~~	~~28~~	~~3b~~	~~R~~	~~F~~	~~C57~~	~~7/17/02~~	~~Norm~~	~~No~~	0	0	0	0	0	0	0	0	0	0
765	28		L	F	C57	7/17/02	Norm	No	1	1	1	0	1	1	1	1	1	0
~~772~~	~~28~~		~~B~~	~~F~~	~~C57~~	~~7/17/02~~	~~Norm~~	~~No~~	0	0	0	0	0	0	0	0	0	0
779	28		N	F	C57	7/17/02	Norm	No	1	1	1	1	1	1	1	1	1	0
786	29a	3a	R	M	C57	7/17/02	High	Yes	1	1	1	0	1	1	1	1	1	0
793	29b		L	M	C57	7/17/02	High	Yes	1	1	1	0	1	1	1	1	1	0
800	29c		B	M	C57	7/17/02	High	Yes	1	1	1	0	1	1	1	1	1	0
807	29d		N	M	C57	7/17/02	High	Yes	1	1	1	0	1	1	1	1	0	1
~~814~~	~~30~~	~~3b~~	~~R~~	~~M~~	~~C57~~	~~7/17/02~~	~~High~~	~~No~~	0	0	0	0	0	0	0	0	0	0
821	30		L	M	C57	7/17/02	High	No	1	1	1	0	1	1	1	1	1	0
828	30		B	M	C57	7/17/02	High	No	1	1	1	0	1	1	1	1	1	0
835	30		N	M	C57	7/17/02	High	No	1	1	1	0	1	1	1	1	0	1
~~842~~	~~31~~	4a	~~R~~	~~F~~	~~C57~~	~~7/24/02~~	~~Low~~	~~Yes~~	0	0	0	0	0	0	0	0	0	0
849	31		L	F	C57	7/24/02	Low	Yes	1	1	1	0	1	1	1	1	0	0
856	31		B	F	C57	7/24/02	Low	Yes	1	1	1	1	1	1	1	1	1	0
863	31		N	F	C57	7/24/02	Low	Yes	1	1	0	0	1	1	1	1	0	0
870	32	4b	R	F	C57	7/24/02	Low	No	1	1	1	0	1	1	1	1	0	0
877	32		L	F	C57	7/24/02	Low	No	1	1	1	0	1	1	1	1	0	0
884	32		B	F	C57	7/24/02	Low	No	1	1	1	0	1	1	1	1	1	0
891	32		N	F	C57	7/24/02	Low	No	1	1	0	0	1	1	1	1	0	0

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Mouse ID	Cage ID	Group	Mark	Sex	Strain	DOB	Diet	Exercise	Scan	Analy.	Mech.	Raman	Scan	Analy.	Mech.	Analy.	Histology	Re-fill
898	33a	4a	R	M	C57	7/24/02	Norm	Yes	1	1	1	0	1	1	1	1	1	0
905	33b		L	M	C57	7/24/02	Norm	Yes	1	1	1	0	1	1	1	1	1	0
912	33c		B	M	C57	7/24/02	Norm	Yes	1	1	1	0	1	1	1	1	0	0
919	33d		N	M	C57	7/24/02	Norm	Yes	1	1	1	0	1	1	1	1	0	1
926	34	4b	R	M	C57	7/24/02	Norm	No	1	1	1	0	1	1	1	1	0	0
933	34		L	M	C57	7/24/02	Norm	No	1	1	1	0	1	1	1	1	0	0
940	34		B	M	C57	7/24/02	Norm	No	1	1	0	0	1	1	1	1	1	0
947	34		N	M	C57	7/24/02	Norm	No	1	1	1	0	1	1	1	1	0	0
954	35	4a	R	F	C57	7/24/02	High	Yes	1	1	1	0	1	1	1	1	1	0
961	35		L	F	C57	7/24/02	High	Yes	1	1	1	1	1	1	1	1	1	0
968	35		B	F	C57	7/24/02	High	Yes	1	1	1	0	1	1	1	1	0	0
975	35		N	F	C57	7/24/02	High	Yes	1	1	1	0	1	1	1	1	0	0
982	36	4b	R	F	C57	7/24/02	High	No	1	1	1	1	1	1	1	1	1	0
989	36		L	F	C57	7/24/02	High	No	1	1	1	0	1	1	1	1	0	1
996	36		B	F	C57	7/24/02	High	No	1	1	1	0	1	1	1	1	0	0
1003	36		N	F	C57	7/24/02	High	No	1	1	1	0	1	1	1	1	0	0
1010	37a	4a	R	F	C57	7/24/02	High	Yes	1	1	1	0	0	0	1	1	0	0
1017	37b		L	F	C57	7/24/02	High	Yes	1	1	1	0	1	1	1	1	1	0
1024	38	5b	R	M	C57	10/28/02	Low	No	1	1	0	0	1	1	1	1	0	0
1031	38		L	M	C57	10/28/02	Low	No	1	1	0	0	1	1	1	1	1	0
1038	38		B	M	C57	10/28/02	Low	No	1	1	0	0	1	1	1	1	1	0
1045	38		N	M	C57	10/28/02	Low	No	1	1	0	0	1	1	1	1	1	0
1052	39a	5a	R	M	C57	10/28/02	Low	Yes	1	1	0	0	1	1	1	1	0	0
1059	39b		L	M	C57	10/28/02	Low	Yes	1	1	0	0	1	1	1	1	0	1
1066	39c		B	M	C57	10/28/02	Low	Yes	1	1	0	0	1	1	1	1	1	0
1073	39d		N	M	C57	10/28/02	Low	Yes	1	1	0	0	1	1	1	1	0	0
1080	40	5b	R	F	C57	10/28/02	Norm	No	1	1	0	0	1	1	1	1	0	0
1087	40		L	F	C57	10/28/02	Norm	No	1	1	0	0	1	1	1	1	0	0
1094	40		B	F	C57	10/28/02	Norm	No	1	1	0	1	1	1	1	1	1	0
1101	40		N	F	C57	10/28/02	Norm	No	1	1	0	0	1	1	1	1	0	0
1108	41	5a	R	F	C57	10/28/02	Norm	Yes	1	1	0	0	1	1	1	1	0	0
1115	41		L	F	C57	10/28/02	Norm	Yes	1	1	0	0	1	1	1	1	0	1
~~1122~~	~~41~~		~~B~~	~~F~~	~~C57~~	~~10/28/02~~	~~Norm~~	~~Yes~~	0	0	0	0	0	0	0	0	0	0
1129	41		N	F	C57	10/28/02	Norm	Yes	1	1	0	0	1	1	1	1	1	0
1136	42	5b	R	M	C57	10/28/02	High	No	1	1	0	0	1	1	1	1	0	1
1143	42		L	M	C57	10/28/02	High	No	1	1	0	0	1	1	1	1	1	0
1150	42		B	M	C57	10/28/02	High	No	1	1	0	0	1	1	1	1	0	0
1157	42		N	M	C57	10/28/02	High	No	1	1	0	0	1	1	1	1	0	0
1164	43a	5a	R	M	C57	10/28/02	High	Yes	1	1	0	0	1	1	1	1	0	1
1171	43b		L	M	C57	10/28/02	High	Yes	1	1	0	0	1	1	1	1	0	1
~~1178~~	~~43c~~		~~B~~	M	~~C57~~	~~10/28/02~~	~~High~~	~~Yes~~	0	0	0	0	0	0	0	0	0	0
1185	43d		N	M	C57	10/28/02	High	Yes	1	1	0	0	1	1	1	1	0	0

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Mouse ID	Cage ID	Group	Mark	Sex	Strain	DOB	Diet	Exercise	Scan	Analy.	Mech.	Raman	Scan	Analy.	Mech.	Analy.	Histology	Re-fill
1192	44	5b	R	M	C3	10/28/02	Low	No	1	1	0	0	1	1	1	1	0	1
~~1199~~	~~44~~		~~L~~	~~M~~	~~C3~~	~~10/28/02~~	~~Low~~	~~No~~	0	0	0	0	0	0	0	0	0	0
1206	44		B	M	C3	10/28/02	Low	No	1	1	0	0	1	1	1	1	1	0
1213	44		N	M	C3	10/28/02	Low	No	1	1	0	0	1	1	1	1	1	0
1220	45	5a	R	M	C3	10/28/02	Low	Yes	1	1	0	0	1	1	1	1	0	1
1227	45		L	M	C3	10/28/02	Low	Yes	1	1	0	0	1	1	1	1	0	0
1234	45		B	M	C3	10/28/02	Low	Yes	1	1	0	0	1	1	1	1	1	0
1241	45		N	M	C3	10/28/02	Low	Yes	1	1	0	0	1	1	1	1	0	0
1248	46	5b	R	F	C3	10/28/02	Norm	No	1	1	0	0	1	1	1	1	0	0
1255	46		L	F	C3	10/28/02	Norm	No	1	1	0	0	1	1	1	1	1	0
1262	46		B	F	C3	10/28/02	Norm	No	1	1	0	0	1	1	1	1	0	1
1269	46		N	F	C3	10/28/02	Norm	No	1	1	0	0	1	1	1	1	1	0
1276	47	5a	R	F	C3	10/28/02	Norm	Yes	1	1	0	0	1	1	1	1	0	0
1283	47		L	F	C3	10/28/02	Norm	Yes	1	1	0	0	1	1	1	1	1	0
1290	47		B	F	C3	10/28/02	Norm	Yes	1	1	0	0	1	1	1	1	0	1
1297	47		N	F	C3	10/28/02	Norm	Yes	1	1	0	0	1	1	1	1	0	0
1304	48	5b	R	M	C3	10/28/02	High	No	1	1	0	0	1	1	1	1	0	1
1311	48		L	M	C3	10/28/02	High	No	1	1	0	0	1	1	1	1	0	0
1318	48		B	M	C3	10/28/02	High	No	1	1	0	0	1	1	1	1	1	0
1325	48		N	M	C3	10/28/02	High	No	1	1	0	0	1	1	1	1	1	0
1332	49	5a	R	M	C3	10/28/02	High	Yes	1	1	0	0	1	1	1	1	0	1
1339	49		L	M	C3	10/28/02	High	Yes	1	1	0	0	1	1	1	1	1	0
1346	49		B	M	C3	10/28/02	High	Yes	1	1	0	0	1	1	1	1	0	1
1353	49		N	M	C3	10/28/02	High	Yes	1	1	0	0	1	1	1	1	1	0
1360	50a	5b	R	F	C57	10/28/02	High	No	1	1	0	0	1	1	1	1	1	0
1367	50a		N	F	C57	10/28/02	High	No	1	1	0	1	1	1	1	1	1	0
1374	50b		R	F	C3	10/28/02	High	No	1	1	0	0	1	1	1	1	0	1
1381	50b		N	F	C3	10/28/02	High	No	1	1	0	0	1	1	1	1	0	1
1388	51	6a	R	F	C57	11/4/02	Low	Yes	1	1	0	0	1	1	1	1	0	0
1395	51		L	F	C57	11/4/02	Low	Yes	1	1	0	0	1	1	1	1	1	0
1402	51		B	F	C57	11/4/02	Low	Yes	1	1	0	0	1	1	1	1	0	1
1409	51		N	F	C57	11/4/02	Low	Yes	1	1	0	0	1	1	1	1	1	0
1416	52	6b	R	M	C57	11/4/02	Norm	No	1	1	0	0	1	1	1	1	0	1
1423	52		L	M	C57	11/4/02	Norm	No	1	1	0	0	1	1	1	1	1	0
1430	52		B	M	C57	11/4/02	Norm	No	1	1	0	0	1	1	1	1	0	0
1437	52		N	M	C57	11/4/02	Norm	No	1	1	0	0	1	1	1	1	0	0
1444	53a	6a	R	M	C57	11/4/02	Norm	Yes	1	1	0	0	1	1	1	1	0	0
~~1451~~	~~53b~~		~~L~~	~~M~~	~~C57~~	~~11/4/02~~	~~Norm~~	~~Yes~~	0	0	0	0	0	0	0	0	0	0
1458	53c		B	M	C57	11/4/02	Norm	Yes	1	1	0	0	1	1	1	1	1	0
1465	53d		N	M	C57	11/4/02	Norm	Yes	1	1	0	0	1	1	1	1	0	1
1472	54	6b	R	F	C57	11/4/02	High	No	1	1	0	0	1	1	1	1	0	1
~~1479~~	~~54~~		~~L~~	~~F~~	~~C57~~	11/4/02	~~High~~	~~No~~	0	0	0	0	0	0	0	0	0	0
~~1486~~	~~54~~		~~B~~	~~F~~	~~C57~~	11/4/02	~~High~~	~~No~~	0	0	0	0	0	0	0	0	0	0
1493	54		N	F	C57	11/4/02	High	No	1	1	0	0	1	1	1	1	0	1

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Mouse ID	Cage ID	Group	Mark	Sex	Strain	DOB	Diet	Exercise	Scan	Analy.	Mech.	Raman	Scan	Analy.	Mech.	Analy.	Histology	Re-fill
1500	55	6a	R	F	C57	11/4/02	High	Yes	1	1	0	1	1	1	1	1	1	0
1507	55		L	F	C57	11/4/02	High	Yes	1	1	0	0	1	1	1	1	0	1
1514	55		B	F	C57	11/4/02	High	Yes	1	1	0	1	1	1	1	1	1	0
1521	55		N	F	C57	11/4/02	High	Yes	1	1	0	0	1	1	1	1	0	1
1528	56	6b	R	F	C3	11/4/02	Low	No	1	1	0	0	1	1	1	1	0	1
1535	56		L	F	C3	11/4/02	Low	No	1	1	0	0	1	1	1	1	0	1
1542	56		B	F	C3	11/4/02	Low	No	1	1	0	0	1	1	1	1	0	1
1549	56		N	F	C3	11/4/02	Low	No	1	1	0	0	1	1	1	1	0	0
1556	57	6a	R	F	C3	11/4/02	Low	Yes	1	1	0	1	1	1	1	1	1	0
1563	57		L	F	C3	11/4/02	Low	Yes	1	1	0	0	1	1	1	1	0	1
1570	57		B	F	C3	11/4/02	Low	Yes	1	1	0	0	1	1	1	1	0	1
1577	57		N	F	C3	11/4/02	Low	Yes	1	1	0	0	1	1	1	1	0	1
1584	58	6b	R	M	C3	11/4/02	Norm	No	1	1	0	0	1	1	1	1	0	1
1591	58		L	M	C3	11/4/02	Norm	No	1	1	0	0	1	1	1	1	1	0
1598	58		B	M	C3	11/4/02	Norm	No	1	1	0	0	1	1	1	1	1	0
1605	58		N	M	C3	11/4/02	Norm	No	1	1	0	0	1	1	1	1	0	1
1612	59	6a	R	M	C3	11/4/02	Norm	Yes	1	1	0	0	1	1	1	1	0	1
1619	59		L	M	C3	11/4/02	Norm	Yes	1	1	0	0	1	1	1	1	0	0
1626	59		B	M	C3	11/4/02	Norm	Yes	1	1	0	0	1	1	1	1	0	0
1633	59		N	M	C3	11/4/02	Norm	Yes	1	1	0	0	1	1	1	1	0	0
~~1640~~	~~60~~	~~6b~~	~~R~~	~~F~~	~~C3~~	11/4/02	~~High~~	~~No~~	0	0	0	0	0	0	0	0	0	0
~~1647~~	~~60~~		~~L~~	~~F~~	~~C3~~	11/4/02	~~High~~	~~No~~	0	0	0	0	0	0	0	0	0	0
~~1654~~	~~60~~		~~B~~	~~F~~	~~C3~~	11/4/02	~~High~~	~~No~~	0	0	0	0	0	0	0	0	0	0
~~1661~~	~~60~~		~~N~~	~~F~~	~~C3~~	11/4/02	~~High~~	~~No~~	0	0	0	0	0	0	0	0	0	0
1668	61	6a	R	F	C3	11/4/02	High	Yes	1	1	0	0	1	1	1	1	0	1
1675	61		L	F	C3	11/4/02	High	Yes	1	1	0	0	1	1	1	1	0	1
~~1682~~	~~61~~		~~B~~	~~F~~	~~C3~~	~~11/4/02~~	~~High~~	~~Yes~~	0	0	0	0	0	0	0	0	0	0
1689	61		N	F	C3	11/4/02	High	Yes	1	1	0	0	1	1	1	1	0	0
1696	62	6b	R	F	C57	11/4/02	Low	No	1	1	0	0	1	1	1	1	0	1
1703	62		L	F	C57	11/4/02	Low	No	1	1	0	0	1	1	1	1	0	0
1710	62		B	F	C57	11/4/02	Low	No	1	1	0	0	1	1	1	1	0	1
1717	62		N	F	C57	11/4/02	Low	No	1	1	0	1	1	1	1	1	1	0
1724	63a	7a	R	M	C57	1/8/03	Low	Yes	1	1	0	0	1	1	1	1	0	1
1731	63b		L	M	C57	1/8/03	Low	Yes	1	1	0	0	1	1	1	1	0	1
1738	63c		B	M	C57	1/8/03	Low	Yes	1	1	0	0	1	1	1	1	1	0
1745	63d		N	M	C57	1/8/03	Low	Yes	1	1	0	0	1	1	1	1	0	1
1752	64	7b	R	M	C57	1/8/03	Low	No	1	1	0	0	1	1	1	1	1	0
1759	64		L	M	C57	1/8/03	Low	No	1	1	0	0	1	1	1	1	0	1
1766	64		B	M	C57	1/8/03	Low	No	1	1	0	0	0	0	1	1	0	1
1773	64		N	M	C57	1/8/03	Low	No	1	1	0	0	1	1	1	1	0	1
1780	65	7a	R	F	C57	1/8/03	Norm	Yes	1	1	0	0	1	1	1	1	0	0
1787	65		L	F	C57	1/8/03	Norm	Yes	1	1	0	0	1	1	1	1	0	1
1794	65		B	F	C57	1/8/03	Norm	Yes	1	1	0	0	1	1	1	1	0	1
1801	65		N	F	C57	1/8/03	Norm	Yes	1	1	0	0	1	1	1	1	1	0

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Mouse ID	Cage ID	Group	Mark	Sex	Strain	DOB	Diet	Exercise	Scan	Analy.	Mech.	Raman	Scan	Analy.	Mech.	Analy.	Histology	Re-fill
1808	66a	7b	R	F	C57	1/8/03	Norm	No	1	1	0	0	1	1	1	1	0	1
1815	66a		L	F	C57	1/8/03	Norm	No	1	1	0	0	1	1	1	1	0	1
1822	66a		B	F	C57	1/8/03	Norm	No	1	1	0	0	1	1	1	1	1	0
1829	66a		N	F	C57	1/8/03	Norm	No	1	1	0	0	1	1	1	1	0	1
1836	66b		R	F	C57	1/8/03	Norm	No	1	1	0	0	1	1	1	1	0	1
1843	66b		L	F	C57	1/8/03	Norm	No	1	1	0	1	1	1	1	1	1	0
1850	67a	7a	R	M	C57	1/8/03	High	Yes	1	1	0	0	1	1	1	1	0	0
1857	67b		L	M	C57	1/8/03	High	Yes	1	1	0	0	0	0	1	1	0	1
1864	67c		B	M	C57	1/8/03	High	Yes	1	1	0	0	1	1	1	1	1	0
1871	67d		N	M	C57	1/8/03	High	Yes	1	1	0	0	1	1	1	1	0	0
1878	68	7b	R	M	C57	1/8/03	High	No	1	1	0	0	1	1	1	1	0	1
1885	68		L	M	C57	1/8/03	High	No	1	1	0	0	0	0	1	1	0	1
1892	68		B	M	C57	1/8/03	High	No	1	1	0	0	1	1	1	1	1	0
1899	68		N	M	C57	1/8/03	High	No	1	1	0	0	1	1	1	1	0	1
1906	69	7a	R	M	C3	1/8/03	Low	Yes	1	1	0	0	1	1	1	1	0	0
1913	69		L	M	C3	1/8/03	Low	Yes	1	1	0	0	1	1	1	1	0	1
1920	69		B	M	C3	1/8/03	Low	Yes	1	1	0	0	1	1	1	1	0	1
1927	69		N	M	C3	1/8/03	Low	Yes	1	1	0	0	1	1	1	1	1	0
1934	70	7b	R	M	C3	1/8/03	Low	No	1	1	0	0	1	1	1	1	0	1
1941	70		L	M	C3	1/8/03	Low	No	1	1	0	0	0	0	1	1	0	1
1948	70		B	M	C3	1/8/03	Low	No	1	1	0	0	0	0	1	1	0	1
1955	70		N	M	C3	1/8/03	Low	No	1	1	0	0	1	1	1	1	0	1
1962	71	7a	R	F	C3	1/8/03	Norm	Yes	1	1	0	0	1	1	1	1	0	1
1969	71		L	F	C3	1/8/03	Norm	Yes	1	1	0	0	1	1	1	1	1	0
1976	71		B	F	C3	1/8/03	Norm	Yes	1	1	0	0	1	1	1	1	0	1
1983	71		N	F	C3	1/8/03	Norm	Yes	1	1	0	0	1	1	1	1	0	0
1990	72	7b	R	F	C3	1/8/03	Norm	No	1	1	0	0	1	1	1	1	0	1
1997	72		L	F	C3	1/8/03	Norm	No	1	1	0	0	1	1	1	1	0	1
2004	72		B	F	C3	1/8/03	Norm	No	1	1	0	1	1	1	1	1	1	0
2011	72		N	F	C3	1/8/03	Norm	No	1	1	0	0	1	1	1	1	0	1
2018	73	7a	R	M	C3	1/8/03	High	Yes	1	1	0	0	0	0	1	1	0	0
2025	73		L	M	C3	1/8/03	High	Yes	1	1	0	0	1	1	1	1	0	1
2032	73		B	M	C3	1/8/03	High	Yes	1	1	0	0	1	1	1	1	0	0
2039	73		N	M	C3	1/8/03	High	Yes	1	1	0	0	1	1	1	1	0	1
2046	74a	7b	R	M	C3	1/8/03	High	No	1	1	0	0	1	1	1	1	0	1
2053	74a		L	M	C3	1/8/03	High	No	1	1	0	0	1	1	1	1	0	1
2060	74a		B	M	C3	1/8/03	High	No	1	1	0	0	1	1	1	1	0	0
~~2067~~	~~74a~~		~~N~~	~~M~~	~~C3~~	~~1/8/03~~	~~High~~	~~No~~	0	0	0	0	0	0	0	0	0	0
2074	74b	7b	R	M	C3	1/8/03	Norm	No	1	1	0	0	0	0	1	1	0	0
2081	74b		L	M	C3	1/8/03	Norm	No	1	1	0	0	1	1	1	1	1	0
2088	75	8a	R	F	C57	1/15/03	Low	Yes	1	1	0	0	1	1	1	1	0	0
2095	75		L	F	C57	1/15/03	Low	Yes	1	1	0	0	1	1	1	1	0	0
2102	75		B	F	C57	1/15/03	Low	Yes	1	1	0	0	1	1	1	1	0	0
2109	75		N	F	C57	1/15/03	Low	Yes	1	1	0	1	1	1	1	1	1	0

Database documenting the animals entered into the studies and progress

Mouse ID	Cage ID	Group	Mark	Sex	Strain	DOB	Diet	Exercise	Scan	Analy.	Mech.	Raman	Scan	Analy.	Mech.	Analy.	Histology	Re-fill
2116	76	8b	R	F	C57	1/15/03	Low	No	1	1	0	0	1	1	1	0	0	0
2123	76		L	F	C57	1/15/03	Low	No	1	1	0	1	1	1	1	1	1	0
2130	76		B	F	C57	1/15/03	Low	No	1	1	0	1	1	1	1	1	1	0
2137	76		N	F	C57	1/15/03	Low	No	1	1	0	0	1	1	1	1	0	0
2144	77a	8a	R	M	C57	1/15/03	Norm	Yes	1	1	0	0	1	1	1	1	0	0
2151	77b		L	M	C57	1/15/03	Norm	Yes	1	1	0	0	1	1	1	1	0	0
2158	77c		B	M	C57	1/15/03	Norm	Yes	1	1	0	0	1	1	1	1	0	0
2165	77d		N	M	C57	1/15/03	Norm	Yes	1	1	0	0	1	1	1	1	1	0
2172	78	8b	R	M	C57	1/15/03	Norm	No	1	1	0	0	1	1	1	1	0	0
2179	78		L	M	C57	1/15/03	Norm	No	1	1	0	0	1	1	1	1	1	0
2186	78		B	M	C57	1/15/03	Norm	No	1	1	0	0	1	1	1	1	0	0
2193	78		N	M	C57	1/15/03	Norm	No	1	1	0	0	1	1	1	1	1	0
2200	79	8a	R	F	C57	1/15/03	High	Yes	1	1	0	0	1	1	1	1	0	1
2207	79		L	F	C57	1/15/03	High	Yes	1	1	0	0	1	1	1	1	0	1
2214	79		B	F	C57	1/15/03	High	Yes	1	1	0	0	1	1	1	1	0	0
2221	79		N	F	C57	1/15/03	High	Yes	1	1	0	0	1	1	1	1	0	1
2228	80	8b	R	F	C57	1/15/03	High	No	1	1	0	0	1	1	1	1	0	1
2235	80		L	F	C57	1/15/03	High	No	1	1	0	0	1	1	1	1	0	0
2242	80		B	F	C57	1/15/03	High	No	1	1	0	1	1	1	1	1	1	0
2249	80		N	F	C57	1/15/03	High	No	1	1	0	0	0	0	1	1	0	0
2256	81	8a	R	F	C3	1/15/03	Low	Yes	1	1	0	0	1	1	1	1	0	0
2263	81		L	F	C3	1/15/03	Low	Yes	1	1	0	0	1	1	1	1	0	0
2270	81		B	F	C3	1/15/03	Low	Yes	1	1	0	0	0	0	1	1	0	0
2277	81		N	F	C3	1/15/03	Low	Yes	1	1	0	0	1	1	1	1	1	0
2284	82	8b	R	F	C3	1/15/03	Low	No	1	1	0	0	1	1	1	1	1	0
2291	82		L	F	C3	1/15/03	Low	No	1	1	0	0	1	1	1	1	0	1
~~2298~~	~~82~~		~~B~~	~~F~~	~~C3~~	1/15/03	~~Low~~	~~No~~	0	0	0	0	0	0	0	0	0	0
2305	82		N	F	C3	1/15/03	Low	No	1	1	0	0	1	1	1	1	1	0
2312	83	8a	R	M	C3	1/15/03	Norm	Yes	1	1	0	0	1	1	1	1	0	1
2319	83		L	M	C3	1/15/03	Norm	Yes	1	1	0	0	1	1	1	1	0	0
2326	83		B	M	C3	1/15/03	Norm	Yes	1	1	0	0	1	1	1	1	1	0
2333	83		N	M	C3	1/15/03	Norm	Yes	1	1	0	0	0	0	1	1	0	0
2340	84a	8b	R	M	C3	1/15/03	Norm	No	1	1	0	0	1	1	1	1	0	0
2347	84a		L	M	C3	1/15/03	Norm	No	1	1	0	0	1	1	1	1	0	0
2354	84a		B	M	C3	1/15/03	Norm	No	1	1	0	0	1	1	1	1	0	0
2361	84a		N	M	C3	1/15/03	Norm	No	1	1	0	0	1	1	1	1	0	0
2368	84b		R	M	C3	1/15/03	Norm	No	1	1	0	0	1	1	1	1	0	1
2375	84b		N	M	C3	1/15/03	Norm	No	1	1	0	0	1	1	1	1	1	0
2382	85	8a	R	F	C3	1/15/03	High	Yes	1	1	0	0	1	1	1	1	1	0
2389	85		L	F	C3	1/15/03	High	Yes	1	1	0	0	1	1	1	1	0	0
2396	85		B	F	C3	1/15/03	High	Yes	1	1	0	0	1	1	1	1	0	0
2403	85		N	F	C3	1/15/03	High	Yes	1	1	0	1	1	1	1	1	1	0

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Mouse ID	Cage ID	Group	Mark	Sex	Strain	DOB	Diet	Exercise	Scan	Analy.	Mech.	Raman	Scan	Analy.	Mech.	Analy.	Histology	Re-fill
2410	86a	8b	R	F	C3	1/15/03	High	No	1	1	0	0	1	1	1	1	0	0
2417	86a		L	F	C3	1/15/03	High	No	1	1	0	0	0	0	1	1	0	1
2424	86a		B	F	C3	1/15/03	High	No	1	1	0	0	1	1	1	1	0	0
2431	86a		N	F	C3	1/15/03	High	No	1	1	0	0	1	1	1	1	1	0
2438	86b		R	F	C3	1/15/03	High	No	1	1	0	0	1	1	1	1	0	0
2445	86b		N	F	C3	1/15/03	High	No	1	1	0	0	1	1	1	1	0	1
2452	87a	9a	R	F	C57	3/19/03	Low	Yes	1	1	0	1	1	1	1	1	1	0
~~2459~~	~~88~~	~~9b~~	~~N~~	~~F~~	~~C57~~	3/19/03	~~Low~~	~~No~~	0	0	0	0	0	0	0	0	0	0
2466	89	9a	N	M	C57	3/19/03	High	Yes	1	1	0	0	1	1	1	1	0	1
2473	90	9b	L	M	C57	3/19/03	High	No	1	1	0	0	1	1	1	1	1	0
2480	91	9a	B	M	C57	3/19/03	Normal	Yes	1	1	0	0	1	1	1	1	0	1
2487			R	M	C57	3/19/03	Normal	Yes	1	1	0	0	1	1	1	1	0	1
2494	92a	9b	R	M	C3	3/17/03	Low	No	1	1	0	0	1	1	1	1	0	0
~~2501~~			~~L~~	~~M~~	~~C3~~	~~3/17/03~~	~~Low~~	~~No~~	0	0	0	0	0	0	0	0	0	0
~~2508~~			~~B~~	~~M~~	~~C3~~	~~3/17/03~~	~~Low~~	~~No~~	0	0	0	0	0	0	0	0	0	0
2515	92b	9b	R	M	C3	3/17/03	Low	No	1	1	0	0	1	1	1	1	1	0
~~2522~~			~~L~~	~~M~~	~~C3~~	~~3/17/03~~	~~Low~~	~~No~~	0	0	0	0	0	0	0	0	0	0
2529			B	M	C3	3/17/03	Low	No	1	1	0	0	1	1	1	1	0	1
2536	93	9a	R	M	C3	3/17/03	Low	Yes	1	1	0	0	1	1	1	1	0	0
2543			L	M	C3	3/17/03	Low	Yes	1	1	0	0	1	1	1	1	1	0
2550			B	M	C3	3/17/03	Low	Yes	1	1	0	0	1	1	1	1	0	0
2557	94a	9b	R	M	C3	3/17/03	High	No	1	1	0	0	1	1	1	1	1	0
2564			L	M	C3	3/17/03	High	No	1	1	0	0	1	1	1	1	0	1
2571			B	M	C3	3/17/03	High	No	1	1	0	0	1	1	1	1	0	1
2578	94b	9b	N	M	C3	3/17/03	High	No	1	1	0	0	1	1	1	1	1	0
2585			B	M	C3	3/17/03	High	No	1	1	0	0	1	1	1	1	0	0
2592	95	9a	R	M	C3	3/17/03	High	Yes	1	1	0	0	1	1	1	1	0	1
2599			L	M	C3	3/17/03	High	Yes	1	1	0	0	1	1	1	1	0	0
2606			B	M	C3	3/17/03	High	Yes	1	1	0	0	1	1	1	1	1	0
~~2613~~	~~97~~	9a	~~R~~	~~F~~	~~C3~~	~~3/17/03~~	~~Normal~~	~~Yes~~	0	0	0	0	0	0	0	0	0	0
2620			L	F	C3	3/17/03	Normal	Yes	1	1	0	0	1	1	1	1	1	0
2627			B	F	C3	3/17/03	Normal	Yes	1	1	0	0	1	1	1	1	0	1
2634			N	F	C3	3/17/03	Normal	Yes	1	1	0	0	1	1	1	1	0	1
~~2641~~	~~98~~	~~9b~~	~~R~~	~~F~~	~~C3~~	~~3/17/03~~	~~Normal~~	~~No~~	0	0	0	0	0	0	0	0	0	0
~~2648~~			~~L~~	~~F~~	~~C3~~	~~3/17/03~~	~~Normal~~	~~No~~	0	0	0	0	0	0	0	0	0	0
2655			B	F	C3	3/17/03	Normal	No	1	1	0	1	1	1	1	1	1	0
2662			N	F	C3	3/17/03	Normal	No	1	1	0	0	1	1	1	1	0	1
2669	99	10a	R	M	C57	3/26/03	Low	Yes	1	1	0	0	1	1	1	1	0	0
2676	101	10a	N	M	C57	3/26/03	High	Yes	1	1	0	0	1	1	1	1	0	1
~~2683~~	~~103~~	~~10a~~	~~R~~	~~F~~	~~C57~~	~~3/26/03~~	~~Low~~	~~Yes~~	0	0	0	0	0	0	0	0	0	0
2690	105	10a	N	F	C57	3/26/03	High	Yes	1	1	0	0	1	1	1	1	0	1

Database documenting the animals entered into the studies and progress

Mouse ID	Cage ID	Group	Mark	Sex	Strain	DOB	Diet	Exercise	Scan	Analy.	Mech.	Raman	Scan	Analy.	Mech.	Analy.	Histology	Re-fill
2697	107	10a	R	M	C3	3/26/03	Normal	Yes	1	1	0	0	1	1	1	1	1	0
2704			L	M	C3	3/26/03	Normal	Yes	1	1	0	0	1	1	1	1	1	0
2711			B	M	C3	3/26/03	Normal	Yes	1	1	0	0	1	1	1	1	1	0
~~2718~~	~~108~~	~~10b~~	~~R~~	~~F~~	~~C3~~	~~3/24/03~~	~~Low~~	~~No~~	0	0	0	0	0	0	0	0	0	0
~~2725~~			~~L~~	~~F~~	~~C3~~	~~3/24/03~~	~~Low~~	~~No~~	0	0	0	0	0	0	0	0	0	0
2732			B	F	C3	3/24/03	Low	No	1	1	0	1	1	1	1	1	1	0
2739			N	F	C3	3/24/03	Low	No	1	1	0	0	1	1	1	1	0	0
~~2746~~	~~109~~	~~10a~~	~~R~~	~~F~~	~~C3~~	~~3/24/03~~	~~Low~~	~~Yes~~	0	0	0	0	0	0	0	0	0	0
~~2753~~			~~L~~	~~F~~	~~C3~~	~~3/24/03~~	~~Low~~	~~Yes~~	0	0	0	0	0	0	0	0	0	0
~~2760~~			~~B~~	~~F~~	~~C3~~	~~3/24/03~~	~~Low~~	~~Yes~~	0	0	0	0	0	0	0	0	0	0
~~2767~~	~~110~~	~~10b~~	~~R~~	~~F~~	~~C3~~	~~3/24/03~~	~~High~~	~~No~~	0	0	0	0	0	0	0	0	0	0
~~2774~~			~~L~~	~~F~~	~~C3~~	~~3/24/03~~	~~High~~	~~No~~	0	0	0	0	0	0	0	0	0	0
2781			B	F	C3	3/24/03	High	No	1	1	0	0	1	1	1	1	1	0
2788			N	F	C3	3/24/03	High	No	1	1	0	0	1	1	1	1	0	1
2795	111	10a	R	F	C3	3/24/03	High	Yes	1	1	0	0	1	1	1	1	0	0
2802			L	F	C3	3/24/03	High	Yes	1	1	0	1	1	1	1	1	1	0
2809			B	F	C3	3/24/03	High	Yes	1	1	0	1	1	1	0	0	1	0
2816			N	F	C3	3/24/03	High	Yes	1	1	0	0	1	1	1	1	0	1
2823	113	11a	R	M	C57	6/4/03	Low	Yes	1	1	0	0	1	1	1	1	0	0
2830			L	M	C57	6/4/03	Low	Yes	0	0	0	0	1	1	1	1	1	0
2837			N	M	C57	6/4/03	Low	Yes	1	1	0	0	1	1	1	1	0	0
2844	115	11a	R	M	C57	6/4/03	High	Yes	1	1	0	0	1	1	1	1	0	1
2851			L	M	C57	6/4/03	High	Yes	1	1	0	0	1	1	1	1	0	0
2858			N	M	C57	6/4/03	High	Yes	1	1	0	0	1	1	1	1	0	0
2865	117	11a	R	F	C57	6/11/03	Normal	Yes	1	1	0	0	1	1	1	1	0	0
2872			L	F	C57	6/11/03	Normal	Yes	1	1	0	0	1	1	1	1	1	0
2879			B	F	C57	6/11/03	Normal	Yes	1	1	0	0	1	1	1	1	1	0
2886			N	F	C57	6/11/03	Normal	Yes	1	1	0	0	1	1	1	1	0	1
2893	119	11a	R	M	C3	6/2/03	Normal	Yes	1	1	0	0	1	1	1	1	0	1
~~2900~~			~~N~~	~~M~~	~~C3~~	6/2/03	~~Normal~~	~~Yes~~	0	0	0	0	0	0	0	0	0	0
2907	120	11b	R	M	C3	6/2/03	Low	No	1	1	0	0	1	1	1	1	0	1
2914			L	M	C3	6/2/03	Low	No	1	1	0	0	1	1	1	1	0	0
2921			N	M	C3	6/2/03	Low	No	1	1	0	0	1	1	1	1	1	0
2928	121	11a	R	M	C3	6/2/03	High	Yes	1	1	0	0	1	1	1	1	0	0
~~2935~~			~~N~~	~~M~~	~~C3~~	6/2/03	~~High~~	~~Yes~~	0	0	0	0	0	0	0	0	0	0
2942	122	11b	R	M	C3	6/2/03	Low	No	1	1	0	0	1	1	1	1	0	0
2949			L	M	C3	6/2/03	Low	No	1	1	0	0	1	1	1	1	0	0
2956			N	M	C3	6/2/03	Low	No	0	0	0	0	1	1	1	1	0	0
2963	123	11a	R	F	C3	6/2/03	Low	Yes	1	1	0	0	1	1	1	1	0	1
2970			L	F	C3	6/2/03	Low	Yes	1	1	0	0	1	0	1	1	0	1
2977			N	F	C3	6/2/03	Low	Yes	1	1	0	1	1	1	1	1	1	0

Database documenting the animals entered into the studies and progress

Mouse ID	Cage ID	Group	Mark	Sex	Strain	DOB	Diet	Exercise	Scan	Analy.	Mech.	Raman	Scan	Analy.	Mech.	Analy.	Histology	Re-fill
2984	124	11b	R	F	C3	6/2/03	Low	No	1	1	0	0	1	1	1	1	0	0
2991			L	F	C3	6/2/03	Low	No	1	1	0	0	1	1	1	1	0	1
2998			B	F	C3	6/2/03	Low	No	1	1	0	0	1	1	1	1	0	0
3005			N	F	C3	6/2/03	Low	No	1	1	0	0	1	1	1	1	0	0
3012	125	11a	R	F	C3	6/2/03	Low	Yes	1	1	0	0	1	1	1	1	0	0
3019			L	F	C3	6/2/03	Low	Yes	1	1	0	0	1	1	1	1	0	0
3026			N	F	C3	6/2/03	Low	Yes	1	1	0	0	1	1	1	1	0	0
3033	127	11a	R	F	C3	6/2/03	Normal	Yes	1	1	0	0	1	1	1	1	0	0
3040			N	F	C3	6/2/03	Normal	Yes	1	1	0	0	1	1	0	0	1	0
~~3047~~	~~128~~	~~12b~~	~~N~~	~~F~~	~~C3~~	~~6/16/03~~	~~Normal~~	~~No~~	0	0	0	0	0	0	0	0	0	0
3054	130	12b	R	F	C3	6/16/03	High	No	1	1	0	0	1	1	1	1	0	0
3061			L	F	C3	6/16/03	High	No	1	1	0	1	1	1	1	1	1	0
3068			N	F	C3	6/16/03	High	No	1	1	0	1	1	1	1	1	1	0
									392	392	128	30	382	381	393	392	120	122

INFLUENCES OF NUTRITION AND PHYSICAL FORCES ON BONE STRUCTURE/FUNCTION PROPERTIES

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INTRODUCTION

Maintenance of bone integrity is dependent on a complex interaction of metabolic (hormones, cytokines, growth factors) and environmental factors (mechanical forces, nutrition availability)[1]. Unfortunately, the specific relationships between these factors and the biomechanical properties of bone tissue remain incompletely quantified. Understanding the influence of nutritional status and mechanical usage on the properties of bone may enhance our ability to prevent stress fractures associated with intense training or fragility fractures accompanying aging bone loss.

The purpose of this study was to investigate the interaction between calcium metabolism and exercise mediated mechanical load on the biomechanical properties of bone.

METHODS

C57BL/6J (n=84 male, n=90 female) and C3H/HeJ (n=69 male, n=67 female) mice were purchased from Jackson Laboratories (Bar Harbor, ME) and separated into groups based on strain, gender, diet, and exercise regime. A synthetic diet consisting of low (0.02% Ca), normal (0.95% Ca), and high (2.0% Ca) basal feed with 10% lactose and 0.67% phosphorus was purchased from Purina Test Diet (Richmond, IN)[2]. An exercise protocol was established and implemented, which consisted of running the mice on a custom built treadmill with a 0° incline. The eight week regime consisted of ramping the speed from 10 to 17 m/min and increasing the duration from 15 to 30 min over the first 4 wks and holding the speed constant for an additional 4 wks[3]. The exercise regime and the controlled calcium diets (low, normal, high) were begun when the animals were 12 weeks old. The mice were humanely euthanized at 20 wks of age, the left femur and eighth caudal vertebrae were dissected free of soft tissue and frozen in LRS. This work was approved by The Animal Care and Use Committee.

Left femora and Cd8 vertebrae were scanned in distilled water on a GE.EVS Micro-CT system. Each scan was reconstructed at a mesh size of 18μm x 18μm x 18μm and a 3D digitized image was generated for each specimen. The femora and vertebrae images were rotated into a standard orientation and thresholded[4]. Geometric analyses were performed on a 3mm mid-diaphysis segment in order to obtain the cross-sectional area, cortical thickness, and moment of inertia (I_{yy}) for each femur. Two standardized volumes of trabecular bone were segmented from the proximal and distal ends of the vertebral bodies for analysis. The bone volume fraction, bone surface to volume ratio, trabecular thickness, trabecular number, trabecular spacing, and the degree of anisotropy were acquired from the vertebral morphologic analyses.

The data was analyzed using SPSS statistical software (Chicago, IL). Multivariate general linear models were used to test for main effects of four factors (gender, strain, diet and exercise) and for interaction effects among the four factors. *Post hoc* tests were used to compare the three levels of dietary calcium. Correlations were considered significant with a p-value less than 0.05.

RESULTS

Femora: Geometric data was collected from 323 femora. Mouse strain had a significant effect on geometric parameters. C57BL/6J mice had a significantly smaller cross-sectional area and cortical thickness as compared to the C3H/HeJ mice. Exercise did not have a significant effect on any of the geometric parameters, as shown in Table 1. Table 2 shows that differences in micro-CT measures by dietary calcium level are not significant.

Table 1: Exercise Effects on Cortical Parameters

Exercise	N	Cross Sectional Area (mm^2)	Cortical Thickness (mm)	Bending I_{yy} (mm^4)
No	168	0.892 (0.20)	0.256 (0.08)	0.116 (0.03)
Yes	155	0.877 (0.20)	0.250 (0.08)	0.114 (0.02)

Data are given as mean (STD)

Table 2: Dietary Effects on Cortical Parameters

Dietary Calcium	N	Cross Sectional Area (mm^2)	Cortical Thickness (mm)	Bending I_{yy} (mm^4)
Low	105	0.891 (0.19)	0.252 (0.08)	0.119 (0.03)
Normal	112	0.884 (0.20)	0.254 (0.08)	0.114 (0.02)
High	106	0.878 (0.21)	0.254 (0.08)	0.112 (0.02)

Data are given as mean (STD)

Vertebrae: Morphologic data was collected from 310 vertebrae. There was no significant effect for exercise on the trabecular parameters, as shown in Table 3. The differences in proximal trabecular parameters for the three dietary calcium groups are shown in Table 4. The normal dietary calcium group had a significantly higher bone volume fraction as compared to the low (p=0.003) and high (p=0.003) groups. The bone surface to volume ratio of the normal group was shown to be significantly lower than the low dietary calcium group (p=0.030), however no significant differences were seen between any of the other groupings. The normal dietary calcium group had a significantly higher trabecular thickness as compared to the low group (p<0.000) and marginally significant as compared to the high (p=0.051) group. Similar results were observed in the distal trabecular parameters.

Table 3: Exercise Effects on Proximal Trabecular Parameters

Exercise	N	Bone Volume Fraction (%)	Bone Surface to Volume Ratio (mm^2/mm^3)	Trabecular Thickness (mm)
No	161	40.9 (0.07)	30.08 (4.57)	0.074 (0.01)
Yes	149	41.9 (0.06)	29.30 (4.44)	0.077 (0.01)

Data are given as mean (STD)

Table 4: Dietary Effects on Proximal Trabecular Parameters

Dietary Calcium	N	Bone Volume Fraction (%)	Bone Surface to Volume Ratio (mm^2/mm^3)	Trabecular Thickness (mm)
Low	101	40.6 (0.07)*	30.29 (4.01)*	0.073 (0.01)*
Normal	109	42.8 (0.07)*	29.18 (5.26)*	0.078 (0.02)*
High	100	40.6 (0.06)*	29.69 (4.07)*	0.075 (0.01)*

Data are given as mean (STD) * Significant, $p \leq 0.05$

DISCUSSION

As expected, the results indicate that geometric properties are dependent on genetic background. C57BL/6J mice had a significantly smaller cross-sectional area and cortical thickness as compared to the C3H/HeJ mice, as noted in previous studies[5].

Differences due to nutritional status were seen only in trabecular bone of the vertebrae, where modeling phenomena are expected to occur more rapidly than in femoral cortical bone due to the greater biodynamic activity of trabecular bone. The loading regimen in this study appears to not have an effect on the femoral geometric or vertebral morphologic properties. The level of exercise did not compensate for the decrease in the trabeacular parameters during dietary calcium alterations. This may be caused by an inadequate load stimulus. Preliminary biomechanical testing data suggests a difference in post-yield behavior in exercise groups. This may possibly suggest an interaction at the tissue level, which we will continue to explore.

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